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PHENOTYPES OF INDIVIDUALS WITH A β THAL CLASSICAL ALLELE ASSOCIATED EITHER WITH A β THAL SILENT ALLELE OR WITH α GLOBIN GENE TRIPLICATION

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ABSTRACT

Background and Objective. β thalassemia intermedia has its origins in compound heterozygosity for many different β thal defects or in an interaction of a β thal defect with altered α cluster. Two specific genetic associations (β thal/β^+ –101 C→T and β thal + ααα or αααα) have been described in recent years as being determining a phenotype similar to that of simple β thal heterozygote or, alternatively, a clinical picture of thalassemia intermedia.

Methods. A detailed study on this subject was carried out on 55 patients divided into 2 groups. Group I consisted of 20 patients, 17 of whom (Group Ia) had a β thal/β^+ –101 C→T genotype and 3 (Group Ib) had a β thal/β IVS II-844 C→G genotype. Group II consisted of 35 patients with β thal association +ααα or $\alpha\alpha\alpha^{anti3.7}$. The methods of study have already been described in a previous issue.[10]

Results. Thirty percent of group Ia and 25% of group II were virtually asymptomatic, while the others presented the thalassemia intermedia phenotype. This second phenotype is generally milder in patients of group I and even less so in those of group II. In the former there is a higher level of HbF; in the second there is more marked α/β+γ globin synthesis imbalance. The severity of the phenotype has no connection with that of the β thal defect. The patients of group Ib all presented thalassemia intermedia.

Interpretation and Conclusions. The definite clinical pictures of groups I and II are quite common in the Italian population and should therefore be well understood, especially for proper application of preventive measures against thalassemia major.

What is now commonly called thalassemia intermedia presents itself like thalassemia major, but is less serious. There are two differences: the patient has a much greater longevity and is largely independent of blood transfusions.

First described in Italy in 1925[1] as a type of constitutional hemolytic icterus (jaundice), this illness was later classified with the anemias.[2] From the beginning, it was observed to come on with variable intensity. Within this range, two levels are clearly recognizable: a serious one, bordering on Cooley's disease, and a mild one, almost identical to the asymptomatic phenotype of heterozygous thalassemia.[2,3] While the study of phenotypic characteristics was carried out only at a hematological level, thalassemia intermedia very often resulted from an evident β thalassemic parent and an apparently normal one. Therefore, for years it was believed that this illness was determined by the heterozygous condition of for thalassemic trait. To explain the more serious condition, compared with the normal asymptomatic one of the heterozygous carrier, the hypothesis of a group of *modifying genes*[3] aggravating classic thal heterozygosis was suggested.

At the same time, discovery of micro-drepanocytic anemia,[4-6] which is very similar to thalassemia intermedia but caused by the association of microcytemia and another hereditary defect, *sickling*, gave

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rise to the hypothesis that the apparently normal parent of an individual with thalassemia intermedia might *always be carrier of another hematological anomaly or something not yet identified,* but hereditary factor, acting with the thalassemic defect.[7]

In later years, improvements in investigative methods on thal defects, especially the application of DNA analysis of globin genes, vastly increased our knowledge on thalassemia intermedia. Now we know we are dealing mostly with homozygotes with slight β thal defects or compound heterozygotes for a serious β thal defect and a slight β defect only recognizable studying the DNA. We also know, however, that in the homozygotes for serious β defects the association with α or γ gene defects reduces the severity of the β thal homozygosis so as to bring it closer to thalassemia intermedia.

Finally, we might be dealing with a double heterozygosis for a β thal defect or an α gene triplication or quadruplication which can greatly upset the globin synthesis balance and the β thal phenotype, thus bringing it closer to thalassemia intermedia.

By developing molecular studies and introducing globin gene investigation techniques, the previous hypothesis that genetic factors attenuate or aggravate the phenotype expression of thalassemia has been confirmed. Simultaneously, it was proved that the parents of thalassemia intermedia patients, previously considered normal, are often carriers of β thal silent allele or α gene triplication.

Nowadays, we know the genotype of about 90% of thalassemia intermedia patients.[8]

While these new frontiers of thalassemia continue to be studied, we can nevertheless start to describe the phenotypes of subjects carrying an obvious β thal defect together with a silent β thal or an α gene triplication. In the previous literature these subjects were diagnosed sometimes simple heterozygous carriers of β thal and sometimes thalassemia intermedia patients.[12-15,17,18-24,31-35] In this article, we present two groups of patients who were entirely studied in our laboratories from the clinical, hematological, hemoglobin and globin synthesis points of view and whose genotype was precisely identified. One group was formed from subjects carriers of β thal defect and –101 C→T silent mutation of the β globin promoter; the other group had double heterozygosis for a β thal defect and an α gene triplication or quadruplication. In addition, we present three cases of compound heterozygosity with an evident β thal defect and a silent IVS II 844 C→G β thal defect.

Materials and Methods

The various phenotypes of 55 subjects were studied in two groups: group I consisted of 20 compound heterozygous subjects with a classic β thal defect and a silent β thal defect. Seventeen of these subjects (Table 1a), 12 females and 5 males, in the 11-66 age range, were compound heterozygotes for an obvious β thal defect and β^+ –101 C→T. These 17 had already been partially studied in a previous article of ours.[9] The other 3 subjects (Table 1b), aged 23, 29, and 36, showed the genotype β° thal/β^+ IVS-II 844 C→G; Group II (Table 2) consisted of 35 subjects, 18 males and 17 females aging from 6 to 75, who had a β thal defect and the triplication or quadruplication of the α gene on one or both chromosomes.

All these patients were examined clinically and subjected to the complete set of hematological, hematochemical, hemoglobin, globin synthesis and molecular tests listed in our previous issue.[10] Serum ferritin was determined in all patients. Traditional techniques were used. The study of the globin genes β, α, and, where necessary, δ and γ was carried out using the techniques already mentioned and the others perfected in our laboratories.[11]

The family trees of many subjects were examined. The relatives, carriers of silent thalassemia or α gene triplication, are included in the list of the above-mentioned issue.[10]

Results

The case history of each subject in the three groups (Ia, Ib, II) is summarized and the results of analysis are given in the Tables. To simplify the examination of each case, information about the spleen is reported in the relative Table. The patients in group Ia and group II are listed in the clinical reports and in tables with the same number, in increasing order of phenotype severity.

Group Ia: compound heterozygotes for a classical β thal defect and the silent β^+ –101 C→T mutation

For the 17 patients of this group, the first and subsequent clinical and genetic diagnoses took place at our center. Some of the patients in the group were identified by screening at school; others in ambulatory screening, more specifically among those apparently healthy carriers of β thalassemia with a marked hematological history; other among the thalassemia intermedia patients, whose DNA study had previously shown only one defect.

A) Clinical findings

1. SF, 17-year-old, from Lazio. Diagnosed at age 14 as β thal carrier following examination of a relative. Later, carrier of two thal defects. Generally excellent health.
2. DCI, 14, from Basilicata. Good health aside from slight pallor.
3. TA, 31, from Abruzzo. Thalassemia diagnosed at school screening. Compound heterozygote for β°39/β^+ –101; pale. Spleen swells 2 cm beyond costal margin. Slight thal-like alterations in cranial bones but no heterotopic bone

Table 1a. Hematologic, hematochemical, hemoglobin and globin synthesis phenotypes of β thal/β^+ –101 C→T carriers.

N.	Pt/sex	Age y.	Hb g/dL	RBC $x10^6$/μL	WBC $x10^9$/L	MCV fL	MCH pg	Morph RBC altered	Ret. ‰ RBC	Erythr. μL	RBC with incl. bodies*	Serum iron μg/dL	Serum ferritin ng/mL	Bilirubin level mg/dL conjug.	Bilirubin level mg/dL total	HbA_2 %	HbF %	α/β+γ ratio	β genotype	Spleen
1	SF/F	17	10.7	5.5	7.3	64	19.6	+++	12	ass.	+	132	150	0.60	2.12	4.0	46.6	1.76	β^+IVS-110/β^+–101	norm.
2	DCl/F	14	10.2	4.6	7.3	65	22.0	+++	6	ass.	++	110	5	0.17	0.81	5.1	9.6	1.60	β^+IVS I-6/β^+–101	norm.
3	TA/M	31	11.8	6.2	5.3	62	19.0	+++	13	ass.	+	94	280	0.26	1.03	5.8	19.1	2.22	β°39/β^+–101	2 cm
4	TM/M	21	12.2	6.6	7.0	61	18.4	+++	3	ass.	+	184	170	0.37	1.27	5.4	20.5	2.16	β°39/β^+–101	norm.
5	RA/M	31	10.1	6.0	5.4	56	16.9	+++	22	54	+++			0.75	3.00	6.1	11.2	1.97	β°39/β^+–101	norm.
6	SA/F	39	10.9	5.6	6.8	60	19.4	+++	9	ass.	ass.	85	35	0..15	0.55	4.9	33.2	1.96	β°IVS II-1/β^+–101	2 cm
7	SS/F	20	10.2	5.6	6.4	59	18.2	+++	6	ass.	ass.	85	45	0.37	1.33	5.9	15.4	2.23	β°IVS I-1/β^+–101	3 cm
8	GM/F	32	9.4	5.8	7.9	54	16.2	+++	12	ass.	+	90	110	0.38	1.04	5.2	21.6	2.33	β°39/β^+–101	3 cm
9	FV/F	11	9.8	5.5	6.0	56	17.7	+++	1	ass.	ass.	108	120	0.48	2.42	5.0	18.4	1.70	β°39/β^+–101	2 cm
10	RN/F	28	8.7	4.6	7.8	59	19.0	+++		ass.		83		0.33	1.10	6.0	11.5		β°39/β^+–101	
11	DP/F	32	9.0	5.0	6.9	60	18.0	+++	15	138	+	88	90	0.48	2.10	4.5	16.7	1.96	β°IVS I-1/β^+–101	3 cm
12	CP/M	26	11.0	7.0	6.8	52	15.8	+++	35	204		77	55	0.49	3.21	6.5	4.2		β^+IVS II-745/β^+–101	5 cm
13	MD/M	50	10.5	5.0	6.2	68	21.3	+++	25	124	++	154	780	0.64	2.00	4.3	37.7	2.11	β°IVS I-1/β^+–101	7 cm
14	MA/F	66	8.4	4.7	7.5	59	17.7	+++	28	300	++	115	380	0.36	1.60	6.1	12.1	2.84	β°IVS I-1/β^+–101	2 cm
15	MA/F	55	7.4	4.3	5.9	56	17.2	++	18	ass.	++	58	180	0.35	1.39	6.4	11.2	2.23	β°IVS I-1/β^+–101	Palp.
16	ML/F	25	7.4	4.7	8.0	57	15.7	+++	22	160	ass.	95	35	0.59	2.59	5.8	18.5	1.97	β°39/β^+–101	3 cm
17	GR/F	37	7.5	3.6	12.4	70	20.7	+++	50	13,640	++++	97	540	0.63	1.78	5.6	19.0	2.22	β°39/β^+–101	Splenect.

RBC osmotic fragility is decreased in all subjects; α genotype is normal in all subjects. Studies to find the –196 C→T and –117 G→A mutation of the A-γ gene promoter have been negative; these results are not reported in the Table.
**+: ~ 10% RBC; ++: ~ 25%; +++: ~ 50%; ++++: ~ 100%.*

Table 1b. Hematologic, hematochemical, hemoglobin and globin synthesis phenotypes of three thalassemia intermedia patients with β thal/β^+ 844 C→G genotype.

N.	Pt/sex	Age y.	Hb g/dL	RBC $x10^6$/μL	WBC $x10^9$/L	MCV fL	MCH pg	Morph RBC altered	Ret. ‰ RBC	Erythr. μL	RBC with incl. bodies	Serum iron μg/dL	Serum ferritin ng/mL	Bilirubin level mg/dL conjug.	Bilirubin level mg/dL total	HbA_2 %	HbF %	α/β+γ ratio	β genotype	Spleen
1	MM/F	29	7.1	3.8	11.6	67	18.6	+++	110	9,280	++++	280	280	0.74	3.10	5.0	18.2	2.27	β°39/β^+IVS II-844	splenect.
2	AE/M	6*	6.9	3.5	6.6	67	19.5							0.50	2.80					
	Idem	23°	11.5	5.6	8.8	69	20.4	++	50	ass	+++	144	2800	0.92	8.00	3.8	3.4	1.84	β^+IVS I-1/β^+IVS II-844	6 cm
3	DLR/F	36	8.3	4.8	7.9	57	17.3	+++	36	158	+++	82	120	0.10	0.40	5.9	9.0	1.82	β^+IVS I-110/β^+IVS II-844	2 cm

RBC osmotic fragility and the α genotype are normal in all subjects. Studies to find the –196 C→T and –117 G→A mutation of the A-γ gene promoter have been negative: these results are not reported in the Table.
*Before transfusion therapy; °Now.

Table 2. Hematologic, hematochemical, hemoglobin and globin synthesis phenotypes of carriers of β thal and triplication or quadruplication of α genes.

N.	Pt/sex	Age y.	Hb g/dL	RBC x10⁶/μL	WBC x10³/L	MCV fL	MCH pg	Morph RBC altered	Ret. ‰ RBC	Erythro-blasts μL	RBC with incl. bodies*	Serum iron μg/dL	Serum ferritin ng/mL	Bilirubin level mg/dL conjug.	Bilirubin level mg/dL total	HbA_2 %	HbF %	α/β+γ ratio	β genotype	α genotype	Spleen
1	VA/F	30	10.3	5.9	6.4	58	17.4	+++	6	ass.	+	91	30	0.10	0.50	5.4	3.4	2.42	β°IVS II-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
2	VP/M	35	12.3	6.7	5.9	58	18.2	++	5	ass.	+	77	175	0.43	2.00	5.8	2.8	2.24	β°IVS II-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
3	AR/M	62	11.9	5.9	6.3	62	20.5	+++				92		0.36	0.89	4.8	4.6		β°IVS I-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
4	AA/M	32	11.9	5.9	7.9	61	20.1	+++				88		0.15	0.75	4.5	5.0	2.28	β°IVS I-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
5	AB/F	29	11.0	5.2	7.0	64	21.2	+++				131		0.16	0.86	4.2	9.6		β°IVS I-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
6	D'AF/M	75	13.5	5.7	12.2	72	23.7	++		ass.		102		0.24	0.96	4.4	2.4		β^{+}IVS II-745/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
7	BS/F	34	11.1	5.7	7.4	61	19.6	+++		ass.		62		0.40	1.36	4.3	5.1		β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
8	KJ/F	26	10.0	5.5	6.4	59	18.2	+++	30	ass.	++	122	40	0.15	0.97	4.5	4.0	2.43	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
9	MM/M	19	12.0	6.1	6.4	61	19.8	+++	8	ass.	+++	42	65	0.47	1.42	5.3	4.9	2.48	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
10	LD/F	30	9.9	5.3	10.2	60	18;6	+++		102		83		0.18	0.85	4.5	6.3		β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	
11	LV/M	57	10.5	5.6	7.5	59	18.7	+++		75		85		0.34	1.23	5.5	5.7		β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	
12	MD/M	22	11.7	6.7	7.6	58	17.6	+++	12	ass.	+++	88	270	0.10	0.54	5.6	3.6	2.21	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	3 cm
13	RM/F	35	10.1	5.4	5.9	61	18.9	+++	10	ass.	++	56	60	0.11	0.54	5.2	5.0	2.47	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	3 cm
14	VC/M	66	10.4	5.6	6.6	61	18.5	+++	14	ass.	++	97	300	0.41	1.71	5.3	2.6	2.44	β°IVS II-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
15	PN/M	27	10.2	5.0	6.6	71	20.6	+++	70	6,600	++++	87	800	0.16	0.78	4.4	3.8	2.70	β°IVS I-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	splenect.©
16	TR/F	22	10.1	5.6	6.0	57	17.9	+++	18	ass.	++	143	490	0.80	5.05	5.2	4.0	2.35	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	3 cm
17	NV/F	11	8.6	4.9	6.1	58	17.6	+++	4	61	++	57	50	0.27	1.05	5.5	2.9	1.42	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
18	DRM/F	67	9.8	5.2	5.7	61	19.0	+++	25	ass.	++	95	270	0.16	0.80	5.0	2.4	2.16	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	3 cm
19	NL/M	39	9.6	5.3	6.9	58	18.3	+++	3	ass.	++	57	100	0.27	1.00	5.0	3.0	2.20	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	5 cm
20	MS/M	6	9.6	5.7	6.0	54	16.8	+++	8	ass.	+++	125	8	0.11	0.45	6.0	4.1	2.41	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
21	MF/M	9	9.4	5.4	8.1	56	17.6	+++	6	ass.	+++	98	20	0.10	0.41	5.0	4.6	3.10	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	norm.
22	CP/F	13	9.3	4.4	6.3	69	20.9	+++	6	63	++	100	190	0.47	1.99	4.7	9.0	2.38	β°IVS I-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	2 cm
23	D'AC/M	46	10.7	4.9	5.6	71	22.0	+++	19		+++	187		0.49	1.59	4.8	3.7	2.40	β^{+}IVS II-745/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	4 cm
24	ZMR/F	40	8.2	4.2	5.4	63	19.5	+++	26	1,134	+++	98	85	0.18	0.96	5.4	2.6	3.00	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	splenect.©
25	SS/F	6	9.2	4.5	5.9	63	20.3	+++	10	118	++	63	175	0.25	0.98	4.8	14.0	2.18	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	5 cm
26	CV/M	45	9.2	4.1	11.6	80	22.2	+++	105	3,016	++++	125	1000	0.54	2.46	4.8	10.2	2.81	β°IVS I-1/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	splenect.
27	TW/M	46	10.6	4.8	4.7	68	21.8	+++	34	141	++	172	1120	0.56	2.05	4.0	18.1	2.42	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	7 cm
28	BN/F	68	9.9	4.0	6.6	76	24.6	++	90	1,980	++++	120	1500	0.57	1.84	2.9	1.9	3.87	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	splenect.
29	BA/M	40	8.8	4.0	15.2	77	22.0	++++	150	14,440	++++	294	750	1.26	3.79	3.1	3.0	3.11	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	splenect.
30	VR/M	60	8.9	3.5	14.0	83		++	60	1,820	++++	251	1400	0.70	2.22	3.0	2.0	3.00	β°39/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	splenect.
31	HH/F	23	8.2	4.5	5.5	60	17.9	+++	40	ass.	+++	69	230	0.48	2.10	5.6	5.5	2.90	β°cod.5/β cd27#	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	3 cm
32	NS/F	45	8.3	4.7	6.9	60	17.7	+++	10	ass.	++	107	290	0.53	2.26	4.8	2.7	2.76	β°IVS I-1/β^{A}	$\alpha\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	4 cm
33	NS/F	43	9.5	4.9	6.6	61	19.3	+++		132		64		0.47	1.41	4.2	4.5		β°IVS I-1/β^{A}	$\alpha\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	4 cm
34	NI/M	11	7.5	4.6	5.8	55	16.3	+++	20	116	++	90	60	1.02	2.15	5.5	2.0	3.19	β°IVS I-1/β^{A}	$\alpha\alpha\alpha\alpha^{anti3.7}/\alpha\alpha^{anti3.7}$	norm.
35	SS/F	59	8.9	3.4	8.0	84	26.1	+++	50	4,800	++++	164	1100	0.70	3.80	3.6	3.0	3.29	β^{+}IVS I-110/β^{A}	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha\alpha^{anti3.7}$	splenect.

RBC osmotic fragility is diminished in all subjects; α genotype is normal in all subjects. Studies to find the −196 C→T and −117 G→A mutation of the Aγ gene promoter have been negative. These results are not reported in the Table.

**+: ~10% RBC; ++: ~25%; +++: ~50%; ++++: ~100%. #See text. ©Splenectomized for other causes.*

marrow masses.

4. TM, 21, brother of TA. Good health but always pale. Physical examination was normal.
5. RA, 31, from Lazio. Pale and very asthenic since childhood. He presents at intervals subicterus and hyperchromic urine. General health is good but coloring is pale yellow. Liver and spleen are not expanded.
6. SA, 39, from Basilicata. Always pale and asthenic. Diagnosed β thal carrier only during pregnancy. Has one healthy son. Spleen swells 2 cm beyond costal margin.
7. SS, 20, from Puglia-Marche. School screening revealed β thalassemia. Compound heterozygote with β° IVS I-1 and β^+ –101. Good health but spleen swells 3 cm beyond costal margin.
8. GM, 32, from Lazio. Has always been in good health. Thalassemia diagnosed at school screening. Compound heterozygote with β° 39/β^+ –101. Two trouble-free pregnancies. Children healthy. Spleen swells 3 cm beyond costal margin.
9. FV, 11, of Lazio-Puglia origin. Always pale and asthenic. Is often subicteric and has hyperchromic urine. Spleen swells 2 cm beyond costal margin.
10. RN, 28, sister of #5. Has always been pale and asthenic. Had normal pregnancy during which our Center diagnosed intermediate thalassemia. One son healthy. After diagnosis has not returned for check-up.
11. DP, 32, of Puglia-Sardinia origin. Pale and asthenic since childhood. Thalassemia diagnosed at school screening. Compound heterozygote with β thal (IVS I-1/β^+ –101). Spleen swells 3 cm beyond costal margin. Cranium and thorax X-ray examination showed slight thickening of parietal bone diploe and absence of paravertebral bone marrow masses.
12. CP, 26, of Lazio-Campania origin. Always very pale and asthenic, sometimes subicteric. Diagnosed at our center at the age of 24. Carrier of cholelithiasis. Spleen swells 5 cm beyond costal margin but not painful.
13. MD, 50, from Lazio. Pale and asthenic. Urine often hyperchromic. Repeated subicteric episodes. Our center diagnosed him at age 48. Liver swells 3 cm beyond costal margin and spleen 7 cm.
14. MA, 66, from Puglia. Healthy until adulthood. Had one normal pregnancy and one healthy son. Had a cholecystectomy for biliary calculosis. First diagnosed for thalassemia intermedia at age 42. Pallid, subicteric and with spleen swelling 2 cm beyond costal margin. Does not have heterotopic bone marrow masses.
15. MA, 55, sister of #14. Has always been very pallid and asthenic since childhood. Examined at the Center after her sister and diagnosed for thalassemia intermedia. Had one normal pregnancy and one healthy son. Pallor is evident. Spleen palpable at costal margin.
16. ML, 25, of Abruzzo-Sardinia origin. Pallid and splenomegalic from childhood. Aged 23, she was diagnosed for thalassemia intermedia at the Center. Very pallid and obviously subicteric. Spleen swells 2 cm beyond costal margin.
17. GR, 37, from Lazio, sister of #8. Pale, splenomegalic and asthenic from childhood. At the age of 18 diagnosed for thalassemia intermedia at our Center, after sister's diagnosis. At 35, her genotype was identified. Has two daughters, both alive and healthy, but she required transfusion therapy during the first pregnancy. At age 31, hypersplenism worsened her anemia and splenectomy required. Since then she continues to be pale and subicteric and has hyperchromic urine. Has no heterotopic bone marrow masses, but slight microareolar thickening of cranial diploe is present. Liver swells 2 cm beyond costal margin.

B) Hematological, hemoglobin, hematochemical and globin synthesis data

This information is presented for each subject in Table 1a.

C) Family studies

In the cases examined, the proband was a son. In 4 cases, both parents were found having a thal defect (obvious β thal or β+ –101). Among all subjects examined there were 4 pairs of brothers who presented an identical clinical pattern (e.g. slight in #3 and 4, more evident in #14 and 15); or different (#5 and 10), or very different, like the two sisters #8 and 17.

Group 1b: compound heterozygotes for a β thal defect and β^+ IVS II 844 C→G mutation

1. MM, aged 29; father from Lazio, thalassemic, carrier of β IVS I-1 mutation; mother from Puglia, apparently non-thalassemic carrier of β IVS II-844 mutation. At 5 diagnosed for β thalassemia; at 24, our Center diagnosed thalassemia intermedia and identified the genetic defects. Has always had Hb 7-7.5 g/dL and clearly jaundiced coloring. At 23, splenectomy (spleen 700 g) and cholecystectomy for biliary calculosis. She had two normal pregnancies, which required BTs. The children are healthy. Always very pale, subicteric, with spleen swelling 2 cm beyond costal margin.
2. AE, aged 23; father from Lazio, thalassemic with β°39 mutation; mother from Puglia, apparently normal but carrier of β IVS II-844 mutation. Aged 6 diagnosed for Cooley's disease, started BT therapy, and then iron chelators. Always pallid, clearly subicteric and hepato-splenomegalic.

At age 21 underwent cholecystectomy for biliary calculosis. Spleen swells 6 cm beyond costal margin. Awaiting splenectomy.

3. DLR, aged 36; both parents from Puglia. Father carrier of β^+ IVS I-110 mutation; mother carrier of β^+ IVS II-844 mutation. Always pale and asthenic. Sent from Policlinico Gemelli, Rome* to our Center for DNA globin study. Has had 2 pregnancies and required some BT during both. General health quite good. Very pale. Liver is not enlarged but spleen swells 2 cm beyond costal margin. Stable at Hb of ~ 8 g/dL not receiving BT.

The hematological, hemoglobin, hematochemical and globin synthesis data for these three subjects are presented in Table 1b.

Group II: heterozygous carriers of a β thal classical allele and an α globin gene triplication or quadruplication

Even in this group the slightest or asymptomatic cases have been identified among those previously classified as simple β thalassemics. Others with recognizable thalassemia intermedia were identified at the Center by specifically searching for gene α triplication, once this defect had already been discovered in other members of the family. A final group of about 10 cases was picked out from the patients with thalassemia intermedia attending our Center for DNA globin analysis.

Of the 35 subjects in this group, only 6 are children or adolescents; the others are young or adults.

A) Clinical findings

1. VA, 36, from Puglia. Always pale but not asthenic. Diagnosed during her one pregnancy which regularly ran. Son healthy. Physical examination negative, but evident pallor.
2. VP, 35, brother of #1. Always very asthenic and pale. Collapsed during military service and was admitted to hospital. Was examined and diagnosed α β thal at our Center after his sister's analysis. Has pallid, subicteric color. Liver and spleen not enlarged.
3. AR, 62, from Emilia. Has always been healthy. Color normal. Liver and spleen not enlarged.
4. AA, 32, son of #3. Member of a couple at risk. Diagnosed at the center in the prenatal diagnosis program. Slightly asthenic for the past several years. Physical examination negative.
5. AB, 29, sister of #4. Always pallid and very asthenic. Normal results in physical examination.
6. D'AF, 75, from Calabria. No illness. Physical examination negative.
7. BS, 34, from Lazio. In good health. Married to a β thal carrier and came to the center for prenatal diagnosis. Has one healthy son. No signs of disease.
8. KJ, 26, of Greek origin. Always pale and asthenic. Has had two normal pregnancies and healthy offspring. Poor general conditions; pale and subicteric.
9. MM, 19, brother of #12. Always pale and subicteric, but not asthenic. Hyperchromic urine. Liver and spleen are not enlarged.
10. LD, 30, of Lazio and Puglia origin. Always pale and asthenic. Diagnosed at our Center but has not returned for checks. Not examined.
11. LV, 57, father of #10. Has never come to the Center nor been examined. Daughter brought a blood sample for analysis and mentioned her father had good general conditions.
12. MD, 22, Sicilian-Piedmont origin. Good general conditions but always pale. Spleen swells 4 cm beyond costal margin.
13. RM, 35, from Campania. Always very asthenic, somewhat pale. Liver swells 2 cm and spleen 4 cm beyond costal margins.
14. VC, 66, father of cases #1 and 2. At 22 admitted to hospital for a long period of jaundice; liver cirrhosis diagnosed. Always pale and evidently subicteric. Hyperchromic urine. Liver and spleen not enlarged. Normal tests of hepatic function.
15. PN, 27, from Abruzzo. Diagnosed at age 7 for β thalassemia and splenomegaly. At 17 underwent splenectomy because of traumatic splenic rupture. At 25, diagnosed for thalassemia intermedia at our Center. Good general conditions. Liver within bounds.
16. TR, 22, From Calabria, daughter of patient #27. Always asthenic, is pale and evidently subicteric; hyperchromic urine, spleen swells 2 cm beyond costal margin, liver not enlarged.
17. NV, 11, daughter of #19. Has always been rather pale. Objective examination negative.
18. DRM, 67, mother of #24. One of sisters of her was anemic and splenectomized at age 36; in her last 20 years of life she was transfusion-dependent. Patient presents pallor, spleen swells 3 cm beyond costal margin, liver not enlarged.
19. NL, 39, from Lazio. Always pale and asthenic, presents remarkable pallor. Liver swells 3 cm, spleen 5 cm beyond costal margins.
20. MS, 6, of Sicilian-Piedmont origin. Poor general conditions. Pale. Liver and spleen not enlarged.
21. MF aged 9, brother of cases #9, 12, and 20. Pale but not asthenic. Liver and spleen not enlarged.
22. CP, 13, from Puglia. Pale and evidently subicteric. Spleen swells 2 cm beyond costal margin; urine always hyperchromic.

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23. D'AC, 46, son of #6. As an adult serious anemia since hemorrhoidal hemorrhages. Pale and subicteric. Liver not enlarged. Spleen swells 4 cm beyond costal margin. Numerous paravertebral intrathoracic bone marrow masses.
24. ZMR, 40, from Campania. Has two brothers, carriers of β thalassemia, both seriously anemic. Always pallid and asthenic since childhood. Two normal pregnancies and healthy children. At age 36 she underwent splenectomy for splenic echinococcosis. Shows intense pallor and is slightly subicteric. Liver not enlarged.
25. SS, 6, from Lazio-Calabria. Poor general conditions, pale, subicteric, liver swells 3 cm., spleen 5 cm beyond costal margins.
26. CV, 45, from Puglia. Pale, asthenic, and splenomegalic since childhood. He is a carrier of HBC antibodies. At age 37 was hospitalized for acute alcoholism. Already checked for cholelithiasis. Emergency splenectomy at age 41, for spontaneous rupture of the spleen. Liver biopsy carried out at the time, showed persistent chronic hepatitis. Another emergency operation, at 44, for perforated piloric ulcer. Carrier of biliary calculosis, always has hyperchromic urine. He is pale, markedly subicteric. Liver consistency normal, 3 cm beyond costal margin. No heterotopic bone marrow masses.
27. TW, 46, from Calabria. Mother anemic and splenomegalic. Always pale and subicteric. Hepatitis B in adolescence. At the age of 30, during hospitalization for jaundice, cirrhogenous chronic hepatitis diagnosis, on the basis of liver biopsy. Shortly afterwards, thalassemia intermedia diagnosis at our Center. Yellowish-brown color for years, urine always hyperchromic, ferritin levels very high (without having BT), and has been undergoing DF therapy. Currently presents enlarged liver up to 2 cm beyond the transverse umbilical line, thinning and hardened at edges, spleen swelling 7 cm beyond costal margin, globose and hard.
28. BN, 68, from Venice. Always pallid, splenomegalic and subicteric. Two normal pregnancies and healthy children. Splenectomy at 30. Due to worsening anemia, she began transfusion therapy at 66. At this moment pale, subicteric, liver swells 2 cm beyond costal margin. X-ray examination shows diffuse osteoporosis, microareolar thickening of cranial diploe but no heterotopic bone marrow masses.
29. BA, 40, from Lazio. At age 7 diagnosis for Cooley's disease and underwent splenectomy. Immediately after, started BT therapy which he interrupted between ages 28 and 36. Subsequently also started ferrochelating therapy with DF by subcutaneous injection. Is pale and evidently subicteric. Liver is firm and palpable on umbilical transverse line, with thinning edges. Gallbladder painful because of certified calculosis. Edema of lower limbs due to cardiac insufficiency. Skeletal thal-like alterations in cranium and long bones. Paravertebral bone marrow masses.
30. VR, 60, from Calabria. Has a very pale, subicteric brother. Splenectomy at 24 because of very large splenomegaly. At 47, our Center diagnosed thalassemia intermedia and he started BT therapy. Always highly subicteric. Has hyperchromic urine. Liver swells 3 cm beyond costal margin. No heterotopic bone marrow masses.
31. HH, 25, Kurd. Has a brother very pale and asthenic. Received occasional BTs. Poor general conditions: pale and subicteric, liver swells 2 cm, spleen 4 cm beyond costal margins. No thal skeletal alterations nor any heterotopic bone marrow masses.
32. NS, 45, from Calabria. Mother and two brothers very pale and asthenic. The patient has been pale and asthenic since childhood as well. Brought a pregnancy to term thanks to numerous BTs. Poor general conditions: pale, subicteric, with liver swells 2 cm beyond costal margin and painful gallbladder. Her spleen is 4 cm beyond the limits and globose. No paravertebral bone marrow masses.
33. NS, sister of #32. In childhood always pallid with recurrent jaundice. Three pregnancies during which BTs were necessary. Poor general conditions, pallor or jaundice; liver swells 2 cm, spleen 4 cm beyond costal margins. X-ray examination showed no cranial bone alterations.
34. NI, 11, son of patient #30. Pale, subicteric, has hyperchromic urine; liver and spleen not enlarged.
35. SS, 59, from Ferrara. Asthenic, pale, subicteric and splenomegalic since childhood. One normal pregnancy and healthy son. At age 42, cholecystectomy for biliary calculosis. Also underwent splenectomy (spleen weight: 4 kg). At age 44, she was diagnosed in our Center as having thalassemia intermedia, and due to serious anemia started BT therapy. Severe pallor, marked jaundice, slightly mongoloid facial features, liver swells 2 cm beyond costal margin. X-ray examination shows thickening of cranial diploe and microareolar osteoporosis, but no thoracic bone marrow masses.

B) Hematological, hemoglobin, hematochemical and globin synthesis data

This information is presented for each subject in Table 2.

C) Family studies

In 13 cases, we were able to study the patient family (Figure 1). In 9 families, one double heterozygote parent transmitted the double heterozy-

gosis to one or more children. In 8 out of these 9 families, the clinical phenotype is very similar in the double heterozygous parent and the children. In one case (the D'AC family), the patient is clearly ill while the father (#6 of Table 2) is a simple thal heterozygote. Out of the 12 not proband children, 7 are carriers of double heterozygosis like the parents, 2 are carriers of β thalassemia, 2 are carriers of $\alpha\alpha\alpha\alpha^{anti3.7}$, and one is entirely free of both defects.

In the other 3 families (MS, RM, BA), both genetic defects are present in one parent and among 10 children (excluding patients), 3 are double heterozygotes, 4 are carriers of only one defect and 3 are entirely free of defects.

Discussion

Group Ia: compound heterozygotes for a classical β thal defect and the silent β^+ −101 C→T mutation

All the subjects in this group have very mild or asymptomatic clinical records, apart from cases 16 and 17 which give a clear picture of thalassemia intermedia. The most frequent disorders are asthenia and pallor from a young age. The most common clinical signs are splenomegaly and subicterus. None of them have ever had BTs, except case #17 during pregnancy. None of the 5 cases that underwent X-ray examination showed intrathoracic bone marrow masses. Two (#3 and 11) of the 7 subjects examined presented slight thalassemic changes in the cranial bones. The hematological picture is typical of marked β thalassemia, though with significantly lower Hb and MCV values (Table 3) than the average in evident β thalassemia (i.e. for Hb: t=6.09, df=86, p<<0.001; for MCV: t=5.78, df=86, p<<0.001). Much more serious alterations in erythrocytic morphology are present. Slight hyperhemolysis is revealed by the presence of reticulocytosis together with a serum bilirubin level above 1 mg/dL. The presence of erythrocytes with inclusion bodies in variable percentage and very unbalanced globin synthesis (ratio α/β+γ mostly between 2 and 2.80) show that there are far too many free α chains and explain the hyperhemolysis, which is a constant feature of these subjects, as due to accelerated destruction of the erythrocytes. Serum ferritin almost always increased in the absence of BT, indicates hyperabsorption of iron from food, as a result of a hyperactive erythropoiesis.

As in all forms of β thalassemia intermedia, HbF level rises in this type also, often to 10-20% and sometimes to 30-40%. This increase does not seem due to γ gene mutation: both the common types in Italy (−196 C→T, −117 G→A of Aγ gene) were absent in all the subjects studied. As in the other β thal intermedia forms and in thalassemia major, this increase is probably a manifestation of the mechanism which produces more γ chains when the β globin chain synthesis, due to the altered β

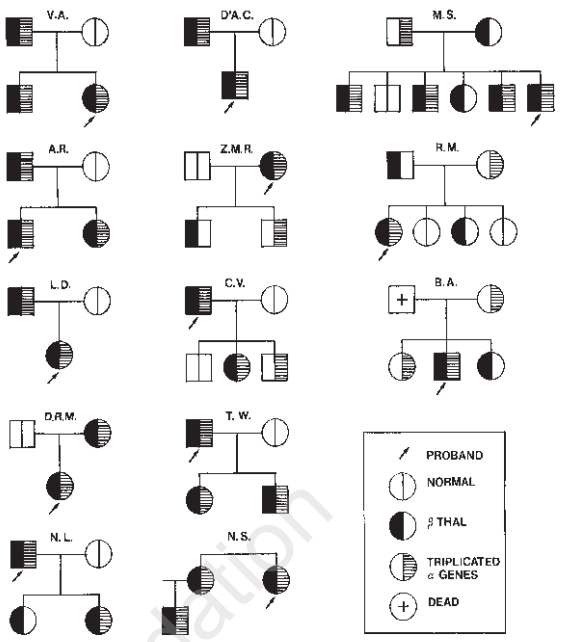

Figure 1. Pedigrees of the families segregating for β thal and a triplicated or quadruplicated α gene complex. Families are indicated with the name of the proband. Only examined subjects were reported.

gene, is reduced or absent. This compensation mechanism probably accounts for the usually slight and steady clinical phenotype in subjects with the β thal/β^+−101 genotype.

One inexplicable detail is the amount of Hb A_2. It tends to be above the average in heterozygous carriers for marked β thalassemia and above the amount in patients with thalassemia intermedia (Table 3). The β thal defect in these subjects is associated with the β^+−101 mutation and is always a serious defect: a nonsense mutation at codon 39; IVS II-1; IVS I-1; IVS-II-110; IVS II-745 mutation. The α genotype is normal in all.

The clinical course is always benign. As already stated, the first diagnosis is usually in adolescence but it may be made during youth or even adulthood. The latest age of the first diagnosis was 37 years. The largely asymptomatic phenotype remains stationary for quite a long time. Increasing age does not seem to aggravate the condition. A moderate splenomegaly can be present, the anemia is slight and the general conditions are satisfactory even into old age. The dominant symptom remains anemia, but this does not alter the patient's quality of life. Women go through pregnancies without much difficulty. Patients are not transfusion-dependent. Length of life does not seem reduced. Only two

Table 3. Average values of some parameters of group Ia (β thal/β^+ –101) and group II (β thal+ $\alpha\alpha\alpha^{anti\ 3.7}$).

Type	N. cases	Hb g/dL	RBC $x10^6$/fL	MCV fL	Hb A_2 %	α/β+γ ratio*
β thal/ β^+ –101 very slight phenotype	5(1)	11.0 ±0.85 ±0.38	5.8 ±0.69 ±0.31	62 ±3.14 ±1.40	5.3 ±0.72 ±0.32	1.94 ±0.23 ±0.11
β thal/ β^+ –101 marked phenotype	12(2)	9.2 ±1.33 ±0.38	5.1 ±0.87 ±0.25	58 ±4.14 ±1.25	5.5 ±0.73 ±0.21	2.16 ±0.30 ±0.09
β thal/ β^+ –101 total number of cases	17(3)	9.7 ±1.47 ±0.36	5.3 ±0.87 ±0.21	59 ±4.17 ±1.04	5.4 ±0.76 ±0.19	2.08 ±0.30 ±0.08
β thalassemia marked phenotype	71	11.8 ±1.23 ±0.15	5.8 ±0.55 ±0.06	65 ±3.77 ±0.45	4.8 ±0.55 ±0.06	1.80 ±0.12 ±0.02
Thalassemia intermedia(4)	20	9.6 ±1.47 ±0.33	4.3 ±0.80 ±0.18	69 ±5.70 ±1.27	4.8 ±0.86 ±0.19	2.22 ±0.17 ±0.05
β thal+ ααα very slight phenotype	8(5)	11.3 ±0.79 ±0.28	5.9 ±0.41 ±0.15	61 ±1.94 ±0.68	4.9 ±0.55 ±0.19	2.37 ±0.09 ±0.04
β thal+ ααα marked phenotype	15(6)	9.9 ±0.82 ±0.21	5.3 ±0.57 ±0.15	61 ±5.10 ±1.32	5.1 ±0.44 ±0.12	2.35 ±0.39 ±0.11
β thal+ ααα severe thalassemia intermedia phenotype	6(7)	9.1 ±1.15 ±0.43	4.1 ±0.48 ±0.18		4.8 ±0.57 ±0.29	2.91 ±0.54 ±0.20
β thal+ ααα total number of cases	29(8)	10.1 ±1.18 ±0.22	5.2 ±0.83 ±0.15	61 ±4.10 ±0.86	5.0 ±0.51 ±0.10	2.57 ±0.41 ±0.09

The average values are followed by the standard deviation and the standard error.

**Subjects examined were: patients with very slight β thal/β^+ –101: 5; patients with serious β thal/β^+ –101: 10; healthy carriers of marked β thalassemia: 24; patients with common thalassemia intermedia: 11; patients with very slight β thal+ ααα: 5; patients with evident β thal+ ααα: 13; patients with severe β thal+ ααα: 6*

1) *Subjects 1-5, Table 1a;*
2) *Subjects 6-17, Table 1a (#17 excluded from the MCV average having had a splenectomy);*
3) *Subjects 1-17 Table 1a (#17 excluded from the MCV average);*
4) *Types of thalassemia intermedia with genotypes different from those considered in this article;*
5) *Subjects 1-9 Table 2 (#6 excluded from all averages because has the phenotype of a healthy simple thalassemic);*
6) *Subjects 10-24 Table 2 (#15 excluded from the MCV average having had a splenectomy);*
7) *Subjects 25-35 Table 2 (excluding the last five, because they have a different genotype; #28, 29, 30 are also excluded from the Hb A_2 average because of having had multiple transfusions). The MCV is not included because only two subjects remain in the group, after excluding the last 5 and those who underwent splenectomy.*
8) *#6, and 31-35 of Table 2 are excluded from all averages, for the reasons above cited. # 15, 24, 26, 28, 29, 30 of Table 2 are excluded from the MCV average because of splenectomy. # 28, 29, 30, are excluded from the Hb A_2 average because of multiple BTs.*

patients (#16 and 17) have a more severe hemolytic anemia which compromises their general health.

A slight β thal intermedia in heterozygotes with a β thal defect and the β^+–101 mutation has already been mentioned in the literature on subjects from Turkey, Bulgaria[12] and some regions of Italy.[13-15] In some of these patients, the phenotype is defined just like that of otherwise healthy carriers of β thalassemia; in others, it is like that of patients with β thalassemia intermedia. Our observations lead us to agree completely with those already reported. Indeed, by means of the analytical examination we performed in the present group, an even more precise distinction between the two phenotypes can be made.

The first 5 on our list (see registers and Table 1), – 30% of the whole group – are clinically asymptomatic. Four of them do not have splenomegaly but 3 have slight increase in serum bilirubin with an average (1.65 mg/dL) which is roughly double that of marked β thalassemics (0.81). A slight increase of reticulocytes is present in 3 out of 5 cases. As for the other hematological parameters, the Hb and MCV are below those of β thal carriers while the erythrocytic morphological changes are more marked. Two characteristics present in all of them are a slightly higher level of HbF and a globin synthesis imbalance with a α/β+γ ratio of about 2. These two facts unequivocably classify apparently healthy subjects among the thalassemia intermedia patients. We therefore do not hesitate in deducing that this variety of β thalassemia intermedia may be suspected in all very marked cases of apparent β thal heterozygotes with a high HbF level and a globin synthesis ratio $\alpha/\beta+\gamma \geq 2$.

The other cases in our list have a pattern of asthenia, pallor, subicterus, and constant though moderate splenomegaly. The average Hb level (9.2 g/dL) is much closer to that of thalassemia intermedia (9.6, Table 3) and in certain cases (e.g. #15, 16, 17) even below 8 g/dL. All subjects but one present reticulocytosis (average 19%), serum bilirubin between 1 and 3 mg/dL, and the HbF level clearly risen. The average α/β+γ globin synthesis ratio is very close to that of thalassemia intermedia (2.16 and 2.22, respectively, Table 3). The clinical and laboratory reports of the last case (#17), a 37-year-old woman who had already had the splenectomy, show the picture of marked β thalassemia intermedia after splenectomy.

There are no differences (Table 1a) in the β genotype between asymptomatic subjects and those with clear thalassemia intermedia. No coinheritance of α cluster or γ gene defect was found to explain the phenotype differences between these patients.

Group Ib – compound heterozygotes for β thal defect and β^+ IVS II-844 C→G mutation

All the subjects with the β thal/β^+ IVS II-844 genotype, even though this defect, completely silent, causes a very slight deficit of β globin chain synthesis,[10] present quite serious thalassemia intermedia. One subject (MM) underwent splenectomy for voluminous splenomegalia at age of 23. The second

(AE), dependent on BT and markedly splenomegalic, will have a splenectomy shortly. All have serious hemolytic anemia. The HbF level has risen only slightly. The α/β+γ globin synthesis ratio is in one case above 2, and in the others at the upper limits of heterozygous β thal.

Case #2 has been attending our center for 22 years and in this long period we could observe the worsening course of the disease.

The case #3 confirms the origin from Puglia that we observed also in our 4 precedent cases.[10] In the literature, there is only one other case[16] of thalassemia intermedia with the IVS II-844 mutation, and that also comes from Puglia.

Group II. Heterozygous carriers of a β thal classical allele and α globin gene triplication or quadruplication

As in group I, the subjects in this second group range from asymptomatic β thalassemics to carriers of very marked β thalassemia or patients with mild or serious β thal intermedia.

The existence of two different phenotypes in carriers of this particular genotype has already been indicated in previous literature. These subjects were described, in some cases, merely as heterozygotes with unusually accentuated β thal hematological characteristics.[17,31,34,35] In other cases, they were considered as patients with evident thalassemia intermedia.[18-20,32,33] In more numerous observations, a distinction was made between asymptomatic thalassemics and patients with thalassemia intermedia.[8,21-24] In a study on 8 patients, the authors even indicated[25] that the incidence of patients with thalassemia intermedia was 25% and that of asymptomatic carriers 75%.

In the present case list, we can classify the first 9 (that is 2.5%) of our register and Table 2 as asymptomatic. They merely seem to be carriers of a clearly thalassemic hematological condition; however, the average values of Hb and MCV are lower than those of even quite marked β thal cases (Table 3); the alterations in erythrocyte morphology are much more serious; reticulocytosis (average 12‰) was frequent as well as various number of erythrocytes with inclusions. The total amount of serum bilirubin was slightly increased, even if it remained below 2 mg/dL. The average level of HbF was 4.9%, i.e. only slightly increased, compared with normal values. Globin synthesis in all subjects was very unbalanced, with α/β+γ ratios between 2.20 and 2.50 and an average of 2.37 significantly higher ($t=10.0$; $df=27$; $p<<0.001$) than that of heterozygous carriers of β thal (1.80) and closer to that (2.22) of patients with thalassemia intermedia.

A second group of 15 subjects (#10-24) presents clear, if not severe, thalassemia intermedia. All cases have long histories of pallor and extreme fatigue. All except the youngest patients and case #17, an old man, have only mild splenomegaly. Two others who were already splenomegalic underwent splenectomy, but for reasons other than thalassemia (car accident and splenic echinococcosis). In addition to evident splenomegaly, #23 presents intra-thoracic masses of heterotopic bone marrow. Hematological pattern is highly thalassemic; anemia is very marked, the average level of Hb is 9.9 g/dL (Table 3), which is significantly lower (11.8) than the majority of serious β thal cases ($t=5.70$; $df=84$; $p<<0.001$) and substantially the same as common thalassemia intermedia, which is 9.6. The number of reticulocytes was slightly higher (average 11.8‰) in all cases and much higher in #15 who underwent splenectomy. Circulating orthochromatic erythroblasts are absent except in this last case. There is a constantly high percentage of erythrocytes with inclusions. Ferritin serum, normal or border-line in some of the younger patients, is slightly increased in the others; the maximum of 800 ng/mL being in a splenectomized subject. In 2/3 of the cases, serum bilirubin is increased but not above 2 mg/dL. Only one case, #16, has an amount up to 5 mg, but she also had both the father (#27) and a brother with high serum bilirubin. This leads us to believe that another hereditary factor could contribute in determining these characteristics. In the patients of this second group the HbF levels are only slightly raised (average 4.3%) or even normal. The α/β+γ globin synthesis ratio is very high (average 2.35), just like the 9 asymptomatic subjects, but has individual values going up as far as 3.00-3.10. One single case (#17), although genotyped β and α just like all the others, has an unexplainable value of 1.42.

One final 11-person subgroup (#25-35 in Table 2) presents rather severe thalassemia intermedia picture. The last 5 (#31-35) have a different genotype and for this reason, we have omitted them in Table 3.

Of the 6 patients numbered 25 to 30, 4 have undergone splenectomy and 2 have slight splenomegaly; 2 present thalassemic bone alterations; 1 has paravertebral bone marrow tissue masses; 3, all adults or elderly depend on BTs, without which they are clearly anemic (Table 2). The hematological characteristics of this group are those typical found in thalassemia intermedia: a slight reticulocytosis in the non-splenectomized patients which increases in the splenectomized patients. In the latter, there is a high percentage of circulamting orthochromatic erythroblasts and inclusions appear in all the erythrocytes. The serum bilirubin is, in most cases, around 2 mg/dL, with a maximum of 3.79 and an average of 2.04. Serum ferritin is moderately increased in a few cases. The amount of Hb A_2 is in the β thalassemia range and only in the transfused patients it is at borderline values. The overall average of Hb F (7.4%), as that of the whole group, is clearly lower than that (34.7%) of patients with classical β thalassemia intermedia. The α/β+γ globin synthesis

ratio is always above 2, and in 4 cases it is between 3.00 and 3.87; the average (2.91, Table 3) is significantly higher than that of the preceding groups (t=2.58; df=17; p≅0.02 for the evident β thal+ααα cases and t=2.19; df=9; p≅0.05 for the very slight cases of β thal+ ααα) and that (2.22) of the thalassemia intermedia cases considered in this work (t=3.98; df=15; p<0.001).

The first 30 cases of Table 2, asymptomatic or with thalassemia intermedia, have a serious thal defect in the β gene (any one of β°39, IVS I-1, IVS II-1, IVS II-745) and an $\alpha\alpha\alpha^{anti3.7}$ in the α cluster. The last 5, however, (#31-35 in Table 2) have different genotypes. Patient #31 (a Kurdish girl) has in the β globin gene a frameshift in the 5th codon, causing β° thalassemia, and a new β chain variant, identified in our laboratories.[36] It is a punctiform C→G mutation in the 27th codon, producing aminoacid ALA→GLY substitution. The abnormal hemoglobin cannot be recognized by either acid or alkalin electrophoresis. The very high (2.99) α/β+γ globin synthesis ratio agrees with that of heterozygotes for a β° thal defect.

Three other subjects (#32-34) from the same family have the β°39 mutation and the $\alpha\alpha\alpha^{anti3.7}$ condition. Patient #35 is heterozygous for β^{+} IVS I-110 and homozygous for $\alpha\alpha\alpha^{anti3.7}$.

The clinical report on all these 5 subjects indicates a thalassemia intermedia as serious as, or perhaps more serious than, the previous cases: it reports anemia, obvious splenomegaly in all cases except an 11-year-old boy, a marked thalassemic hematological pattern, constant hyperhemolysis, slight increase of serum ferritin; and very unbalanced globin synthesis. It is highly probable that each subject's genotype affects the degree of symptoms expressed. This is true particularly in the case of #31, which presents two β gene defects, and in other cases, with 6 instead of 4 α genes (in 3 cases $\alpha\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$ and in one case $\alpha\alpha\alpha^{anti3.7}/\ \alpha\alpha\alpha^{anti3.7}$).

The only case of β thal and $\alpha\alpha\alpha^{anti4.2}$ mentioned in literature,[26] showed a thalassemic hematological pattern. All the cases[27-30] with β thal defect and $\alpha\alpha\alpha^{anti3.7}/\alpha\alpha\alpha^{anti3.7}$ showed an evident thalassemia intermedia with hemolytic anemia, moderate hepatosplenomegaly, pronounced imbalance of globin chain synthesis with a α/β+γ globin synthesis ratio higher than 3.5, low amounts of HbF, benign course of the illness, and all the other characteristics of our case. It is highly probable that this phenotype depends on the more pronounced globin chain synthesis imbalance, caused by a larger number of α genes functioning normally. A globin synthesis ratio much above 1 is common to all cases in literature.

In conclusion, it is clear that the presence of α gene triplication more often gives rise to a clinical pattern of thalassemia intermedia than to an asymptomatic one. In our admittedly approximate classification only 25% of cases are of the latter type, even if many parameters are often more pronounced than in carriers of classical β thalassemia. All the others in group II have an evident phenotype of thalassemia intermedia with some particularly marked parameters: for example, the average α/β+γ globin synthesis ratio (2.57, Table 3) is significantly higher than that (2.22) of patients with common thalassemia intermedia (t=2.71; df=33; p≅0.01).

One distinguishing characteristic of this illness is that the level of HbF, with rare exceptions (5 out of 35 cases), rises very little. The average level of 5% is nowhere near the 34.7% of thalassemia intermedia with other genotypes considered in this and other studies.[31,32]

In this group the family histories show that, in at least 6 families, other family members probably have the same illness as the patient; and the family studies, accomplished in 13 patients, show (Figure 1) that in 10 families both the genetic defects were transmitted from one parent to the son, and in 3 other families, there was one anomaly in each parent. In these 3 families the children are, as expected: 3 double heterozygotes; 3 exempt from both defects, 4 carriers of one defect only.

As in group Ia, in group II members of the same family with identical genotypes may have phenotypes of varying severity. Patient #23, has hemolytic anemia with splenomegaly and paravertebral bone marrow masses; the father, having an apparently identical genotype, has a simple β thalassemia picture; the mother, completely examined for all defects of β and α clusters, proved negative.

We do not yet know why there is this great variety of phenotypes in carriers of the β thal allele +ααα. A correlation has been suggested[18,32] between the degree of gravity of the phenotype and that of the β thal defect. This has not been confirmed by the observations of other authors nor by our own studies, adduced here. All the cases, both serious and mild, present the same β thal defects.

In some cases of β thal+ ααα association, no deletion defects in an α gene of the normal chromosome or of the ααα chromosome were found,[17] which reduce the α/β globin synthesis imbalance and therefore weaken the clinical severity. However, these defects were absent in all of our numerous cases. A different capacity to remove the surplus α chains has been suggested.[24] Our observations permit another hypothesis: there is, in these cases, a clear correlation between the severity of the α/β+γ globin synthesis imbalance and the severity of the phenotype; and another study of ours[10] shows that even among the heterozygous carriers of α triplication, 25% have an absolutely normal α/β ratio and 75% have more or less markedly >1 ratio. These data lead us to believe that the cluster ααα can produce different amounts of α chains. This variable ααα globin synthesis activity can cause differences in the clinical severity of cases with this genotype.

The extra α gene may therefore function at times, and other not: the causes of this difference have not been cleared yet.

The relatively large number of cases we have observed and the uniformity of study methods also allow us to make an initial comparison between the phenotypes of thalassemia intermedia taken into consideration.

Leaving aside the cases with the β thal/β^+ IVS II-844 genotype, which present evident thalassemia intermedia that worsens with age, the other two groups (Ia and II) both have either an asymptomatic or a thalassemia intermedia phenotype. Even though the percentage of asymptomatic cases is basically the same in both groups (about 30% in the subjects with β thal/β^+ –101 and about 25% in those with β thal+ ααα), the remaining differ markedly in the severity of their clinical manifestations. The association of the β thal with the β^+ –101 allele actually gives rise to rather slight phenotypes which progress benignly, only 2 of the 17 being evident thalassemia intermedia cases (#16 and 17). The β thal+ ααα association, on the other hand, often progresses much more seriously, and the patient more often becomes BT-dependent in adulthood or old age. Of the various parameters, there are two which are strikingly different in these two groups: (i) the α/β+γ globin synthesis imbalance, which is more marked in the subjects with β thal+ ααα than in those with β thal/β^+ –101 (2.57 vs 2.08; p<0.001); (ii) the level of HbF is much higher in subjects with β thal/β^+ –101 (average 15.2%) than in those with β thal+ ααα (average 5.0%). In the former, the globin synthesis imbalance caused by impaired output of β chain is partially by the synthesis of γ chain and the consequent production of some HbF. These two features explain the generally mild clinical phenotype. In the second, the impairment of β chain synthesis is not compensated by anything; indeed, it is made worse by the increased activity of the triple α cluster. All of these factors cause a more serious α/β+γ globin synthesis imbalance than that of the β thal/β^+ –101 variety. The result is an additional damage because of endocellular precipitation of free α chains and a more serious clinical picture.

The present survey underlines the genetic associations of an evident β thal defect with a silent one or an α gene triplication or quadruplication. These genetic conditions are quite common in determining a picture of thalassemia intermedia classical or, more frequently, mild or silent.

For example,[8] it was estimated that the β thal+ ααα associations explain 40% of thalassemia intermedia cases in which previously only one β thal molecular defect was identified.

The high incidence of these very mild types of thalassemia intermedia, including very mild forms, also poses an ethical problem:[9] whether or not to interrupt a pregnancy when the fetus carries one of these genotypes. If it is indeed true that in adulthood or old age some patients can develop an overt picture of thalassemia intermedia, it is also true that in about 1/3 of the cases the phenotype remains all life long that of simple heterozygous β thalassemic and this reality cannot be ignored.

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